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CONDITIONS FOR KEEPING LABORATORY ANIMALS IN 100-LITER INOCULATION CAGES DURING LONG-TERM CHRONIC EXPERIMENTS

(Uslovia soderzhaniya laboratornykh zhivotnykh
v 100-litrovykh zatravochnykh kamerakh
pri dlitel'nom khronicheskom eksperimente)

V. A. Popov

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CONDITIONS FOR KEEPING LABORATORY ANIMALS IN 100-LITER INOCULATION CAGES DURING LONG-TERM CHRONIC EXPERIMENTS

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This work deals in greater detail and more systematically than our previous study with several microclimate factors of the widely used today 100-liter inoculation cages; the effect of these factors can to one degree or another affect the results of the experiment.

One of the basic life products of animals in inoculation cages is ammonia (Fraser et al.). Therefore, we considered it important as an indication of the degree of air contamination by volatile life products of the animals in the cages. These products include oxygen and carbon dioxide, ammonia, hydrogen sulfide, methane, indole, skatole, amines, amides, aldehydes and ketones, mercaptans, disulfides and other compounds (Burnett; Merkex et al.). Concentrations of these products in the air depend on the kind of animal, the number per unit of floor area and the frequency of air exchange, as well on the kind of bedding material.

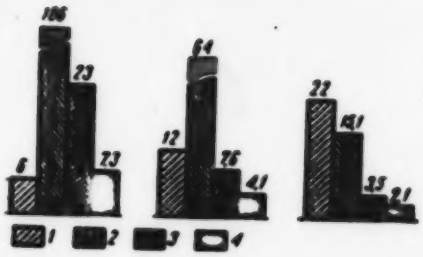
We used inoculation cages with 5-17 rats, weighing an average of 200 g, kept on the usual sawdust bedding. The rate of the air supply into the cages was changed in stages, 10, 20-25 and 35-60 l/min., which corresponded to air exchanges of 6, 12 and 22, on the average.

The dynamics of the accumulation of ammonia in a cage with 17 rats between daily bedding changes with various air exchanges are as follows. The lowest concentration is noted 3-5 hours after cleaning, then an increase is observed in the amount of ammonia, at the end of a 24-hour period reaching 172 mg/m^3 with 6-fold air exchange, 147 mg/m^3 with 12-fold and 38 mg/m^3 with 22-fold, which is that usually maintained during experiments. Mean daily concentrations

of ammonia in cages with 17, 10 and 5 rats with various ventilation conditions are shown in the illustration. When the air exchange was increased from 6 to 22 times and the number of rats reduced from 17 to 5, the mean daily amount of ammonia in the cage fell from 106 to 2.1 mg/m^3. Under the best ventilation conditions, with the usual load of 17 rats, the mean daily concentration of ammonia in the cage is 15 mg/m^3 (daily variations from 4 to 38 mg/m^3). With irregular bedding changes, such as can happen on days off, the ammonia content in the cage increases 2-4 times.

The presence in the air of inoculation cages of such high biologically-active (I. G. Kalinin; V. M. Nizhegorodov and I. G. Kalinin) concentrations of ammonia, sometimes exceeding the level of the chemical substances being studied in the cages by 4 or even 5 orders, is objectionable for a number of reasons. First of all, against this background of ammonia, it is impossible to set and determine accurately concentrations of many chemical substances, as ammonia, a highly reactive compound, interacts with several of these substances in the air of the cage (ozone, sulfur gas, acids, etc.) with the formation of new compounds which can eventually change the response reaction of the animals. Ammonia, changing the pH of the absorbing medium in sampling the substance under study, as well as interacting with reagents used to analyze the samples, distorts the analysis of the air medium for a number of substances (hydrogen sulfide, amines, amides, nitriles, oxidants, etc.).

Secondly, ammonia can change the reactivity of the test animals to the substances in question (Fraser et al.). The high tone of ammonia in the cages probably makes it entirely impossible to study the biological effect of atmospheric concentrations of related substances or those determined by the ammonia ion (amines, amides, nitriles). The most unfavorable aspect is the wide variability in the ammonia background in the cages during 24 hours (10-fold range of variations), so that its changing effect is evidently not systematic and, therefore, cannot be corrected in analyzing results of the experiment. Also, these fluctuations can increase the variance in biological indices, which are not distinguished by great constancy in rats as it is. It must also be taken into consideration that ammonia is probably not the only component of the mixture of volatile life products of rats in the cages.



Mean daily ammonia content (mg/m^3) in the air of a 100-liter inoculation cage with various air exchanges and number of rats.

1--air exchanges in cage; 2--ammonia content in cage with 17 rats (mg/m^3); 3--ammonia content in cage with 10 rats (mg/m^3); 4--ammonia content in cage with 5 rats (mg/m^3).

U. Leyn-Petter indicates that sawdust bedding is a poor sorbent of urine. We decided to replace this bedding with peat, which is being used increasingly in zoohygiene (V. N. Ivanov). Peat is a good binder of the ammonia in animal urine, and being quite acid, hinders the activity of bacteria breaking down the urine to ammonia, as well as having a number of additional advantages over other kinds of bedding.

We tested peat bedding in the form of pressed slabs (produced by the Kalinin branch of the All-Union Scientific Research Institute of the Peat Industry) and crumbs in a 5 cm thick layer on the bottom of the cages. When pressed slabs were used, the concentration of ammonia in the cages remained constant for 3 days, averaging 0.15 mg/m^3 (variations from 0.07 to 0.22 mg/m^3). On the 4th day it increased to 6-8 mg/m^3. With the use of peat crumbs, the concentration of ammonia in the cage remained at an average level of 0.82 mg/m^3 (variations from 0.64 to 1 mg/m^3) for 2 days and on the 3rd day increased to 17-24 mg/m^3. Unlike the situation when sawdust bedding is used, reducing the frequency of air exchange in the cages with peat from 22 to 6 does not essentially affect the ammonia level. This is important, as with 22-fold air exchange, usually maintained to prevent the accumulation of ammonia the extremely high rate of air movement by itself has an unfavorable effect on the test animals, which are subject to colds, especially in uncontrolled temperature conditions.

It has been established, therefore, that the cause of high ammonia contamination of the air in inoculation cages is keeping the animals on sawdust bedding. Replacing it with peat bedding (made of pressed peat) sharply re-

duces the ammonia content 2 orders and the cages need be cleaned only once every 3 days. The dustiness of the air in the cage is the same for peat and sawdust bedding (from 2.2 to 7.7 mg/m^3). Humidity in the cage with peat bedding averages 17% (variations from 8 to 26%) lower than with sawdust bedding.

Besides the high degree of ammonia contamination, 100-liter inoculation cages have a number of other drawbacks. Tests with a visible aerosol of ammonium chloride showed that air from the cages escapes primarily through loose joints in walls, bottom, covers and doors, and not through the drawoff tube, unsuccessfully located in the upper cover alongside the inlet. This leads to contamination of room air. It is difficult to suggest a rational scheme of air supply and removal for this cage design. In more modern vertical cages, air is fed into the upper part and removed at the bottom, using forced exhaust. With 2 conical covers, this provides uniformity of distribution of the test substances (including aerosols) throughout the cage (Hinners et al.).

Studying temperature-humidity regimens in inoculation cages, we found that intracage humidity is determined by the humidity of the outside air, but exceeds it by 20-30%. 100% humidity is often noted in cages. Intracage temperature is also, to a certain degree, determined by outside temperature. In summer it can reach 30°, and in winter 20-22°, which, however, does not exclude the possibility of the local chilling effect on the animals of a stream of outside air when, as is often observed, it is fed into the cages at almost floor level. Reducing the number of rats to 10, and especially to 5, improves the microclimate in the cages and makes it possible for the floor area allotted per animal to approach the upper limit of recommended optimum (300 cm^2); but this does not meet the requirement to increase the number of test animals to obtain representative data.

Such a contradiction cannot be solved with these cages.

Different authors make different recommendations with regard to optimum parameters of the temperature-humidity regimen for keeping rats. For temperature, the suggested optimum varies from 16 to 25°, and for humidity from 40 to 65% (V. N. Ivanov; Yu. I. Lifshitz; Fraser et al.). Fraser et al., emphasize the close relation between the toxic effect of substances and temperature and humidity of the medium; this is also related to functional changes in external

respiration of the animals. This indicates the imperative need to maintain these parameters of the medium at a constant level, independent of marked variations in outside air, i.e. the necessity of conditioning the air fed into the cages.

Summarizing results of the study, we can state that although the changes we suggest will greatly improve the conditions of keeping test animals, nevertheless, they cannot essentially solve the problem of finding better experimentation techniques in sanitary toxicology, as the design of 100-liter cages itself has become very obsolete.

Various institutes are using different designs of inoculation cages in toxicological experiments and there are no common requirements for the keeping of test animals in them.

Conclusions

1. High humidity and a high ammonia content are created in the air of 100-liter inoculation cages with 17 rats kept on sawdust bedding. In the presence of other disadvantages, this makes the cages unsuitable for the hygienic standardization of atmospheric pollutants.

2. The use of peat bedding reduces the ammonia content in the air of the cages an average of 2 orders, as well as decreases the humidity.

3. To improve conditions of keeping laboratory animals during an experiment, it is recommended that more modern cages be designed.

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